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Tosteson, Thomas Robert and Yamamura, Yasuhino(Ponce School of Medicine)

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The ELIZA method initially employed in this project, using immune chicken IgG coated plates did not appear to be specific for microbial adhesion enhancing macromolecules (MAEM). Hyperimmune mouse IgG made against MAEM (but not normal mouse IgG) reacted very strongly with assay plates which were either pretreated with 2% gluteraldehyde (GLA) or coated with immune chicken (egg) immunoglobin(s), in the presence or absence of AAE antigens. Assays yielded titers of approximately 100,000 for the hyperimmune mouse sera over that of the normal control. The very high titers of hyperimmune mouse serum suggested immunization had produced an antibody(ies) against MAEM antigens, however, the assay could not discriminate specific from nonspecific reactions. 28 hybridoma clones have been shown to produce reactive IgG. The ELIZA assay has been refined by repeatedly absorbing the antiserum with GLA pretreated

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DATE: 6 MARCH 1990

FINAL REPORT ON CONTRACT N00014-88-K-0131

TITLE: The Molecular Specificity of Adsorption of Biofilm Macromolecules and Accumulation of Microbial Biofouling on Artificial Surfaces in the Sea.

PRINCIPAL INVESTIGATORS: T.R. Tosteson and Y. Yamamura. Department of Marine Sciences, University of Puerto Rico-Mayaguez and Microbiology Department, Ponce School of Medicine, Ponce, Puerto Rico.

RESEARCH OBJECTIVES: The diversity and variability of macromolecular components that mediate initial microbial attachment to surfaces in ambient seawater is being determined employing immunological techniques. Antisera against microbial adhesion enhancing macromolecules (MAEM) have been raised in chickens and the immunoglobin-G (IgG) fraction utilized for isolation of crude MAEM from samples of cell free coastal seawater and marine microbial culture media by immunoaffinity chromatography. The objective of the present study is to produce monoclonal antibodies (MAbs) against MAEM and use these to chromatographically isolate and purify individual MAEM from mixtures of such components. Variability and diversity of MAEM produced by biofouling microorganisms, those found soluble in ambient seawater and on biofouled surfaces will be assessed employing a spectrum of MAEM MAbs. The specificity of the interactions of the various MAEM with glass and metallic surfaces will be assessed. Alterations in the physical characteristics of the test surfaces and changes in their susceptibility to microbial biofouling will be correlated with these interactions.

PROGRESS TO DATE:

As previously reported the ELIZA method using immune chicken IgG coated plates did not appear to be specific for microbial adhesion enhancing macromolecules (MAEM). Hyperimmune mouse IgG made against MAEM (but not normal mouse IgG) reacted significantly with coated plates in the presence or absence of MAEM antigens. Assays yield titers of approximately 100,000 for the hyperimmune mouse sera over that of the normal control. Hyperimmune mouse sera consistently showed this property despite modifications in the assay method. The very high titer of hyperimmune mouse serum suggests that immunization has produced an antibody(ies) against MAEM antigens, however, the assay could not discriminate specific from nonspecific reactions. To date, 28 hybridoma clones have been shown to produce reactive IgG. These clones were stored frozen in liquid nitrogen, until the ELISA assay could be refined.

1. Refinement of the ELISA procedure to detect an anti-MAEM antibody.

Previous results indicated that anti-MAEM mouse hyperimmune sera reacted very strongly with assay plates which were either pretreated with 2% gluteraldehyde (GLA) or coated with immune chicken (egg) immunoglobin(s), in the presence or absence of AAE antigens. Since the chicken antibody most likely would be selective for the AAE subcomponents it bound, it was decided initially to further explore the usefulness of a GLA method. Experiments utilizing GLA pretreated polyvinyl chloride (PVC) plates were focused primarily on ; (1) reducing the background reactivity (i.e. reactivity seen in the absence of MAEM

antigens) of the hyperimmune mouse serum, and (2) to then determine the optimum concentration of MAEM for ELISA screening of anti-MAEM monoclonal antibodies.

Either of the ELISA methods described previously might have been acceptable but for the strong background reactivity of the immune serum. Attempts were made to reduce the background reactivity by repeatedly absorbing the antiserum with GLA treated PVC plates. In the first series of experiments, 96 well PVC assay plates were treated with 2% GLA at room temperature for 60 minutes and then blocked with 2% BSA for an additional 60 minutes at the same temperature. The plates were washed and 100 ul aliquots of hyperimmune mouse serum, diluted 1/1,000 in fetal bovine serum (FBS) were placed in each well. Inoculated plates were incubated at 37° C for 60 minutes, following which the aliquots were transferred to corresponding wells of identically prepared PVC plates. These procedures were repeated 8 times and the average reactivity (i.e. the amount of nonspecifically bound mouse IgG) of each plate was assessed by a direct immunoperoxidase ELISA. Repeated absorption with GLA treated PVC progressively reduced the amount of mouse IgG that bound to the plastic (Figure 1), however, this procedure did not significantly affect the titer of anti-MAEM antibody, as shown in Figure 2. The anti-MAEM titration was performed at a concentration of 100 ng MAEM/ml. In order to optimize the ELISA titration of anti-MAEM antibody, GLA treated PVC plates were coated with serial dilutions of MAEM antigen , before blocking with BSA. The optimal ELISA reaction was obtained at a MAEM concentration of 50 ng/ml, range of from 40 to 60 ng/ml (Figures 3a and 3b).

Results indicate; (1) that repeated absorption of hyperimmune serum with GLA treated PVC effectively removed nonspecific IgG fractions from hyperimmune mouse serum without changing the titer of anti-MAEM activity, (2) the refined ELISA method using GLA-PVC pre-absorbed hyperimmune serum demonstrated that GLA treated PVC could effectively bind MAEM and (3) the MAEM concentration to be used for coating plates is very critical (optimum concentration 50 ng/ml) and higher concentrations of MAEM did not necessarily bind the antibody more effectively. Attempts to reduce the background activity of the hyperimmune mouse serum by repeated absorption with plates coated with MAEM immune chicken IgG have so far been unsuccessful. The absorption procedure reduced both the background (i.e. in the absence of MAEM) and MAEM mediated antibody reactivities.

The bulk preparation of pre-absorbed hyperimmune mouse serum has been carried out using 3 ml of 1/100 FBS diluted serum applied to the back side of GLA treated PVC plates and absorbed for 4 hours on a rotating platform. Renewed hybridoma screening has begun utilizing the refined ELISA method described above. Renovation of the animal facility at the Ponce School of Medicine has been completed and breeding of BALB/c mice to be used in the next phase of this study has been reinstated from a newly purchased group of inbred mice.

2. Isolation of AAE Macromolecules from Coastal Seawater and Microbial Culture Media.

Microbial adhesion enhancing macromolecules (MAEM) have been recovered from samples of coastal seawater and culture media of laboratory grown microalgae, initially isolated from these waters. Macromolecular constituents in particle free aliquots of these samples were adsorbed to hydroxylapatite (HAP) and subsequently eluted from the HAP at high ionic strength (0.3 to 0.4 molar NaCl). Macromolecules in this fraction of the high molecular constituents of coastal seawater and microbial media have been shown to have all the AAE activity present in these samples (Tosteson, T.R. et al., Journal of Colloid and Interface Science 104:60-71, 1985). The molecular size distribution of HAP

purified materials from these two sources was determined using ultrafiltration membranes (Amicon).

Results indicated that HAP purified MAEM in the microalgal media were fairly evenly distributed in the size categories examined (Figure 4). HAP purified MAEM recovered from seawater samples was not evenly distributed in these size categories. The absence of material > 100k daltons, as well as the increased amounts found in the 100k - 30k dalton size range compared to that found in microalgal cultures may reflect the fact that the size distribution of MAEM in seawater samples would be a function of both their removal by particulate sedimentation as well as production, while in microalgal culture media the major process reflected would be the production of these materials. The microbial adhesion enhancement activity (MAE) of these polymeric macromolecules has been reported in each of the size ranges noted above (T.R. Tosteson, Proc. 7th Inter. Cong. Mar. Corr. Biofoul., Valencia (Spain), 1988). The MAE activity of the newly isolated macromolecules in each these size ranges is being confirmed. The different molecular weight fractions are being examined using the refined ELISA method described above, in order to determine which fraction(s) the mouse antiserum will recognize as the antigen.

INVENTIONS: None.

PUBLICATIONS AND PRESENTATIONS:

Adhesion in the Marine Environment. T.R. Tosteson. Symposium on Adhesion in Biological Systems, joint meeting of the American Society for Cell Biology and the American Society of Biochemistry, San Francisco, January 29-February 3, 1989.

Marine Microbial Polysaccharides and Ocean Thermal Energy Conversion (OTEC). T.R. Tosteson. Department of Chemical Engineering, University of Puerto Rico-Mayaygüez, Mayagüez, Puerto Rico. February 24, 1989.

Molecular Specifity of Microbial Adhesion Enhancing Macromolecules. T.R. Tosteson and Yasuhiro Yamamura. Presented by Y. Yamamura at the Marine Biosurfaces Contractors Meeting, May 20-22, 1989. Hopkins Marine Station, Pacific Grove, California.

TRAINING ACTIVITIES:

Ms. Angela Ramsey, a recently enrolled Ph.D. student is being trained in the use of the Light Sectioning Microscope for determination of wet film thickness on surfaces exposed to ambient coastal seawater and solutions of MAEM.

AWARDS AND FELLOWSHIPS:

T.R. Tosteson received a Scholarly Productivity Award from the Puerto Rico NSF-EPSCOR Program in October, 1989.

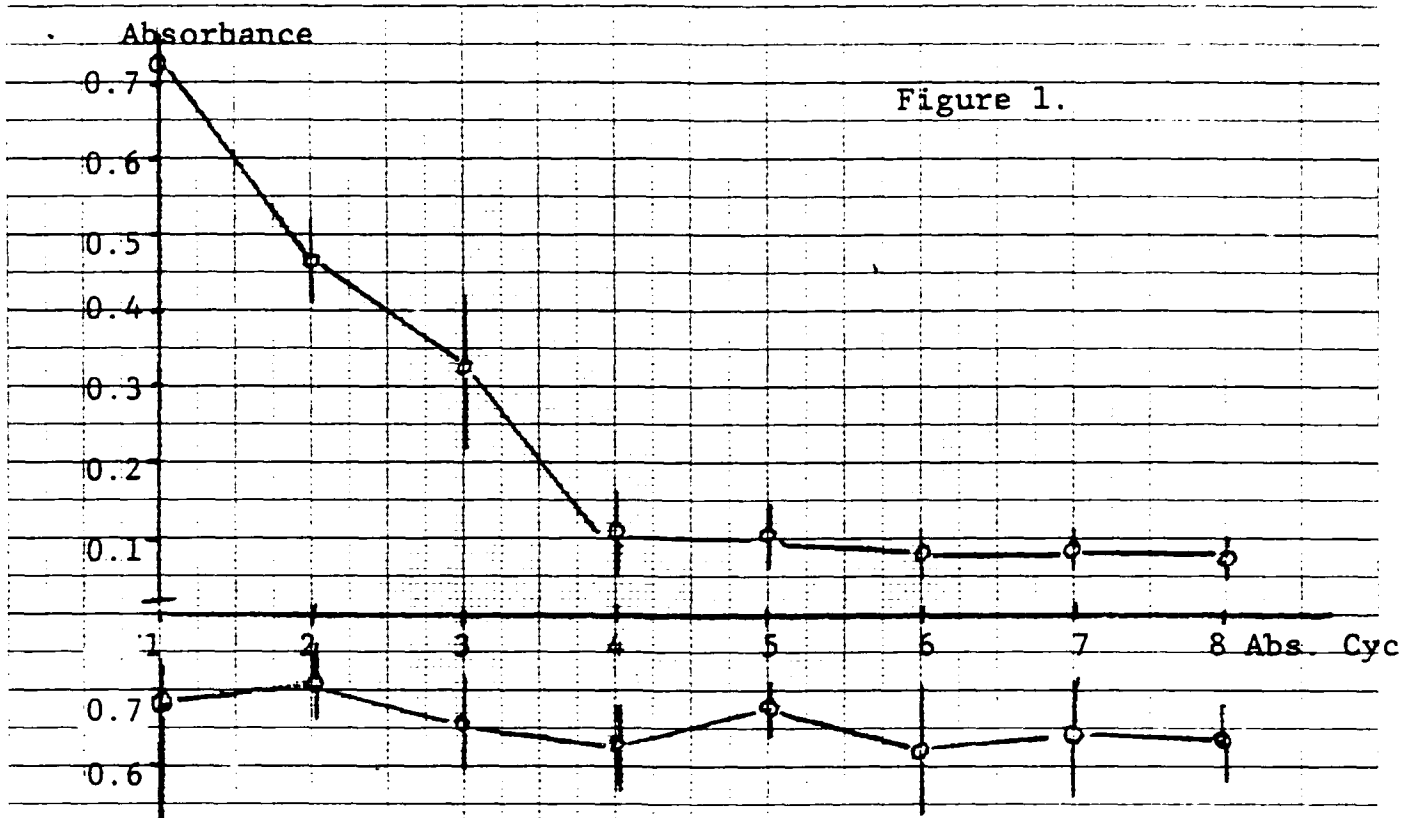

Figure 1.

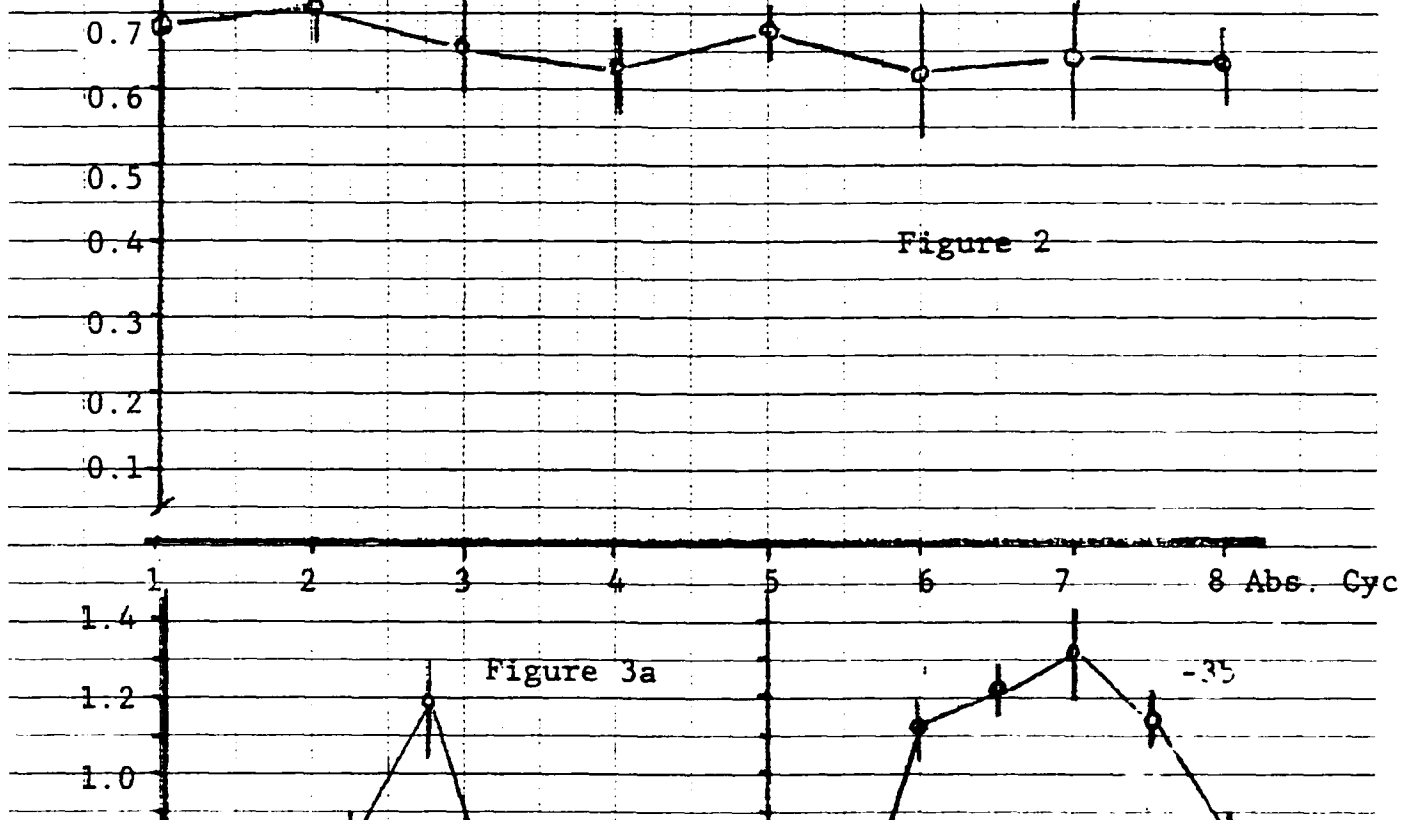

Figure 2

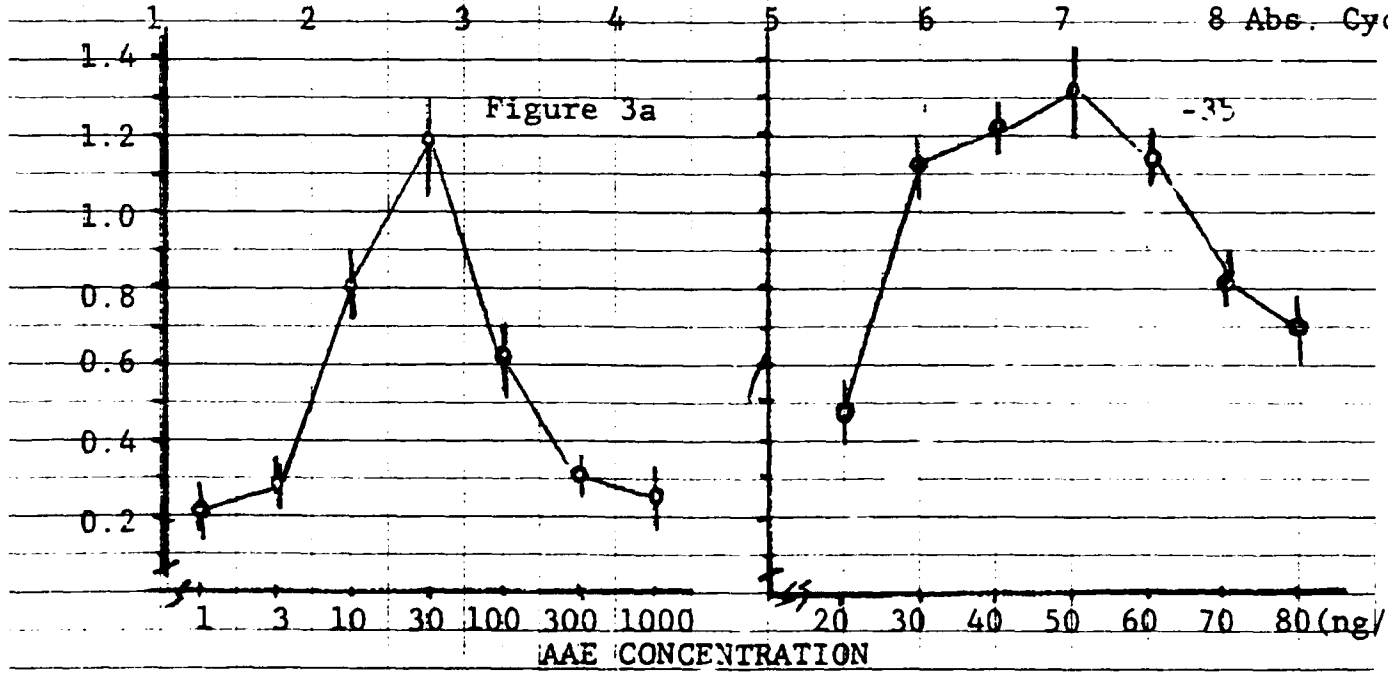

Figure 3a

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FIGURE 4.

HAP PURIFIED AAE ANTIGENS: DISTRIBUTION OF MOLECULAR SIZE

MOLECULAR SIZE (DALTONS)	% TOTAL ANTIGENS	
	LUMINESCENT BAY	MICROALGAL CULTURE MEDIA
>100,000	7	38
100,000-30,000	59	25
30,000-10,000	34	37